

## SUPPLEMENTAL FIGURES

**Figure S1. Conditional ablation of Wnt7b results in smaller offspring but proper hair follicle placode patterning.** (A-D') Embryonic pup phenotype in YFP+ Con and Wnt7b KO transgenic mice. YFP+ Wnt7b cKO transgenic mutant pups were consistently smaller in size than Con littermates. YFP reporter expression revealed normal primary (arrow) and secondary (arrowhead) patterning of HF placodes in Con (E, F, G, H) and Wnt7b cKO (E', F', G', H') YFP+ dorsal skin. (I) Quantification of HF numbers in E18.5 and P2 Con and Wnt7b cKO pups. (J) Dorsal and ventral views of Con (left) and Wnt7b cKO (right) mice at P10 and 1 year (J') of age demonstrating smaller overall body size of Wnt7b cKO mice compared to littermate controls.

**Figure S2. Wnt7b-deficient mutant mice display proper epidermal differentiation marker expression and functional epidermal barrier integrity.** Immunofluorescence detecting epidermal differentiation marker expression in E18.5 YFP+ Con (A, B) and YFP+ Wnt7b cKO (A', B') dorsal back skin. Normal expression of K1 (red), a marker of suprabasal cells in the epidermis was observed in YFP+ Con (A) and YFP+ Wnt7b cKO epidermis (A'). Proper expression of loricrin (red) was observed in the upper, differentiating layers of YFP+ Con (B) and YFP+ Wnt7b cKO (B') epidermis. DAPI (blue) counterstaining was used to label all nuclei in immunofluorescent images. Scale bar = 50µm.

**Figure S3. Wnt7b-deficiency during hair follicle development affects downstream effectors of canonical Wnt signaling.** Immunohistochemistry (IHC, purple) detecting nuclear Lef-1 (A-C') in Con (A, B, C) and Wnt7b cKO (A', B', C') HF's at P2 (A-A'), P5 (B-B') and P8 (C-C') during HF development. IHC (purple) detecting  $\beta$ -catenin (D-F') in Con (D, E, F) and Wnt7b cKO (D', E', F') HF's at P2 (D-D'), P5 (E-E') and P8 (F-F') during HF development. Scale bar = 50µm.

**Figure S4. Wnt7b ablation results in delayed hair follicle regeneration following hair follicle cycle synchronization.** (A-E') Analysis of skin pigmentation and hair re-growth in the waxed region of Con (A-E) and Wnt7b cKO (A'-E') dorsal back skin. (F-H') Macroscopic views of biopsies taken 10 days post-waxing (pw) and 14 days pw (I, I') demonstrating Con and Wnt7b cKO hair follicle regeneration.

**Figure S5. Longterm Wnt7b cKO hfSCs ability to effectively repopulate the “new” hair follicle bulges during hair follicle regeneration.** (A) Schematic representing RU486 treatment timeline used in the second, prolonged telogen (P43-P59) to efficiently YFP+ label HF in Wnt7b cKO mice. (B) At 1 year of age, Wnt7b cKO HF display abundant YFP+ labeled hfSCs in both the “old” and “new” bulge regions (B). Scale bar = 50µm.

**Figure S6. Comparison of proteins homology between Wnt7a and Wnt7b.** (A) Mouse Wnt7a (349 AA) and Wnt7b (353 AA) protein sequences. (B) Sequence alignment comparing mouse Wnt7a with mouse Wnt7b demonstrating approximately 76% sequence homology.